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NIDN-10439

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: I. Henriksen, et al. Group Art Unit: To be assigned
Serial Number: 10/071,505 Examiner: To be assigned
Filing Date: February 8, 2002
Title: Administering a Gravity Segregation Dispersion by Continuous Infusion

Completion of Claim for Priority

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants hereby submit the official certified copy of the priority document number **GB 9920392.9** in connection with the above identified application, benefit of which is claimed in the declaration of this application. The Examiner is most respectfully requested to acknowledge receipt of this certified copy in the next Official Office Action.

Respectfully submitted,

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1.	Your reference	8.3.70981		
2.	Patent application number (The Patent Office will fill in this part)			
3.	Full name, address and postcode of the or of each applicant (underline all surnames)	Nycomed Imaging AS Patent Department Postboks 4220 Torshov N-0401 Oslo Norway		
	Patents ADP number (if you know it)			
	If the applicant is a corporate body, give country/state of incorporation	Norway 0737 3889001		
4.	Title of the invention	Improvements in or relating to diagnostic imaging		
5.	Name of your agent (if you have one)	Frank B. Dehn & Co.		
	"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	179 Queen Victoria Street London EC4V 4EL		
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11.

Frank B. Deane

I/We request the grant of a patent on the basis of this application.

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Improvements in or relating to diagnostic imaging

5 This invention relates to the administration of
dynamic, particulate, dispersion systems, e.g. gas-
containing diagnostic contrast agents, more particularly
to apparatus and a method for the controlled and
substantially steady state administration of such
10 gravity segregating dispersions by infusion.

 In the field of ultrasonography it is well known
that contrast agents comprising dispersions of gas
microbubbles are particularly efficient backscatterers
of ultrasound by virtue of the low density and ease of
15 compressibility of the microbubbles. Such microbubble
dispersions, if appropriately stabilised, may permit
highly effective ultrasound visualisation of, for
example, the vascular system and tissue
microvasculature, often at advantageously low doses of
20 the contrast agent.

 Gas-containing contrast media are also known to be
effective in magnetic resonance (MR) imaging, e.g. as
susceptibility contrast agents which will act to reduce
MR signal intensity. Oxygen-containing contrast media
25 also represent potentially useful paramagnetic MR
contrast agents.

 In the field of X-ray imaging gases such as carbon
dioxide may be used as intravascular contrast agents.
Moreover, the use of radioactive gases, e.g. radioactive
30 isotopes of inert gases such as xenon, has been proposed
in scintigraphy, for example for blood pool imaging.

 Gas-containing ultrasound contrast agents are
usually administered intravenously as a single or
multiple bolus dosage, leading to a rapid and pronounced
35 but relatively short lived rise in backscatter intensity
in respect of blood-perfused tissue and organs as the
bolus mixes with surrounded blood and is carried through

the circulation system. A plot of backscatter intensity against time therefore shows a relatively narrow and high signal intensity peak; backscatter measurements are normally made during the existence of this peak, although this may give rise to problems in, for example, the imaging of deeper tissue and organs where high backscatter from overlying tissue may cause excessive shadowing during the peak period.

As discussed in WO-A-9748337, diagnostic artefacts such as shadowing may be reduced by controlling the rate of administration of the contrast agent and/or by administering a flush such as normal saline after administration of the contrast agent. Contrast agent administration rates of $1-8 \times 10^6$ vesicles/kg-sec or 1×10^{-7} to 3×10^{-3} cc gas/kg-sec and flush rates of 0.01-2.4 ml/sec are suggested; the contrast agent is typically administered over a period of 5-20 seconds, and any subsequent flush is typically administered over a period in the range 10 seconds to 10 minutes.

Continuous infusion of ultrasound contrast agents, for example over a period in the range from one minute to one hour, is of potential interest in that it may permit administration of the contrast agent at a rate which minimises diagnostic artefacts such as shadowing and may lengthen the useful time window for imaging beyond the relatively short duration of the backscatter signal peak resulting from passage of a contrast agent bolus.

Thus, for example, Albrecht et al. in Radiology 207, pp. 339-347 (1998) note that the use of continuous contrast agent infusion to provide prolonged enhancement of Doppler signals is advantageous in that it may permit completion of lengthy imaging procedures such as studies of the renal arteries or peripheral leg veins and may optimise dose effectiveness of the contrast agents, as well as reducing saturation artefacts.

Administration of contrast agents by infusion may

also be useful in procedures based on imaging of contrast agent in the recirculating phase following admixture with the blood pool, as described in WO-A-9908714.

5 A problem with the continuous infusion of gas-containing diagnostic contrast agents arises from the tendency of the gas-filled components to float, since this will lead to inhomogeneities forming within vessels such as power-driven syringes which may be used to
10 administer the contrast agent. This may, for example, lead to an increase in microbubble concentration in the upper part of such a vessel and/or to changes in size distribution occurring at various points within the vessel as larger microbubbles float more rapidly than
15 smaller microbubbles.

 A possible solution to this problem is proposed in WO-A-9927981, which discloses powered injector systems comprising a syringe which is subjected to rotational or rocking motion in order to maintain homogeneity within
20 the contents thereof. In specific embodiments the barrel of the syringe is positioned horizontally in contact with wheels or moveable brackets which are capable of alternately rotating the syringe in opposite directions about its longitudinal (i.e. horizontal) axis.

25 It will be appreciated that the incorporation of such rotational or other agitational means into syringe driver apparatus necessarily complicates the design and significantly increases the cost of such apparatus, so that there is an ongoing need for apparatus which
30 permits the continuous infusion of gas-containing ultrasound contrast agents while maintaining substantial homogeneity of the contrast agent.

 The present invention is based on the finding that controlled delivery of substantially homogeneous gas-
35 containing contrast agent may be achieved by an infusion procedure in which the contrast agent is delivered from an essentially vertically positioned syringe, or other

cylinder shaped reservoir, e.g. tubing and is co-administered to a subject with a flushing medium.

By using an essentially vertically positioned reservoir, e.g. a syringe, as opposed to the horizontal orientation normally employed in e.g. syringe drivers, the height of the contrast agent sample in the syringe is greatly increased, thereby extending the distance through which flotation may occur. Since microbubbles of a given size will rise through carrier liquid at a constant rate, this significantly reduces the effects of flotation separation and thereby improves dose control over a given period of time.

Co-administration of the contrast agent with a flushing medium further enhances product homogeneity, e.g. by reducing residence time and thereby the effects of flotation in connecting tubing etc., and permits particularly efficient control of administration of the contrast agent since the flow rates of both the contrast agent and flushing medium may be independently controlled.

Co-administration also reduces the need for dilution of the contrast agent, this being favourable as contrast agents are often unstable when stored after dilution. Moreover, by co-administering contrast agent with the flushing medium, the presence of an open injection route, independent of contrast agent flow and local blood flow variations is ensured.

Thus according to one aspect of the present invention there is provided a method of administering a gas-containing contrast agent to a subject by continuous infusion, wherein said contrast agent is controllably delivered from an essentially vertically positioned reservoir, e.g. a syringe and is co-administered with a flushing medium to the subject.

According to a further aspect the invention provides apparatus useful in the administration of a gas-containing contrast agent by continuous infusion,

said apparatus comprising (i) syringe driver means adapted to retain a contrast agent-containing syringe in an essentially vertical position and to controllably expel contrast agent therefrom; (ii) mixing means to
5 allow co-administration of said expelled contrast agent with a flushing medium; and (iii) conduit means for conducting said diluted contrast agent to an injection device.

The term "essentially vertical" as used herein
10 denotes that the longitudinal axis of the syringe should be positioned within about 30° of vertical, preferably within 15° and more preferably within 5° of vertical. The syringe may be positioned for either upward or downward delivery of contrast agent. In the former case
15 such flotation as occurs during administration of the contrast agent will tend to lead to a reduction in microbubble concentration as administration proceeds. Conversely the microbubble concentration will tend to increase during administration in the latter case. In
20 either case this may, if desired, be counteracted by appropriate adjustment of the rate at which the contrast agent is administered with flushing medium. Also, it is envisaged that the essentially vertical reservoir, e.g. syringe, may be flipped at a suitable stage during
25 infusion.

Syringe driver means which may be used in accordance with the invention include power injection systems in which the syringe plunger is controllably driven by an appropriate automated mechanism, for
30 example an electrically powered and controlled helical screw or push rod.

The rate at which the contrast agent is administered may, for example, be in the range 0.01-0.25 ml/minute, and may be selected to take account of
35 factors such as the microbubble concentration and the desired degree of attenuation in ultrasound studies.

The flushing medium may be any appropriate

biocompatible liquid, but is preferably normal saline. It may, for example, be administered by gravitational flow using appropriate flow rate controlling means, or may be delivered using a controllable pump. Flow rates
5 of 1-2 ml/minute, have been found to be appropriate although higher flow rates may also be used.

Mixing of the contrast agent and flushing medium may, for example, be effected in a three way connector or tap which is also connected to an injection device
10 such as a needle or catheter. It is preferred that connections are made using low volume tubing in order to minimise transit time of contrast agent and thus to minimise the potential for flotation separation of microbubbles.

15 Gases which may be present in contrast agents administered in accordance with the invention include any biocompatible substances, including mixtures, which are at least partially, e.g. substantially or completely, in gaseous or vapour form at the normal
20 human body temperature of 37°C. Representative gases thus include air; nitrogen; oxygen; carbon dioxide; hydrogen; inert gases such as helium, argon, xenon or krypton; sulphur fluorides such as sulphur hexafluoride, disulphur decafluoride or trifluoromethylsulphur
25 pentafluoride; selenium hexafluoride; optionally halogenated silanes such as methylsilane or dimethylsilane; low molecular weight hydrocarbons (e.g. containing up to 7 carbon atoms), for example alkanes such as methane, ethane, a propane, a butane or a
30 pentane, cycloalkanes such as cyclopropane, cyclobutane or cyclopentane, alkenes such as ethylene, propene, propadiene or a butene, and alkynes such as acetylene or propyne; ethers such as dimethyl ether; ketones; esters; halogenated low molecular weight hydrocarbons (e.g.
35 containing up to 7 carbon atoms); and mixtures of any of the foregoing. Advantageously at least some of the halogen atoms in halogenated gases are fluorine atoms;

thus biocompatible halogenated hydrocarbon gases may, for example, be selected from bromochlorodifluoromethane, chlorodifluoromethane, dichlorodifluoromethane, bromotrifluoromethane, 5 chlorotrifluoromethane, chloropentafluoroethane, dichlorotetrafluoroethane, chlorotrifluoroethylene, fluoroethylene, ethylfluoride, 1,1-difluoroethane and perfluorocarbons. Representative perfluorocarbons include perfluoroalkanes such as perfluoromethane, 10 perfluoroethane, perfluoropropanes, perfluorobutanes (e.g. perfluoro-n-butane, optionally in admixture with other isomers such as perfluoro-iso-butane), perfluoropentanes, perfluorohexanes or perfluoroheptanes; perfluoroalkenes such as 15 perfluoropropene, perfluorobutenes (e.g. perfluorobut-2-ene), perfluorobutadiene, perfluoropentenenes (e.g. perfluoropent-1-ene) or perfluoro-4-methylpent-2-ene; perfluoroalkynes such as perfluorobut-2-yne; and perfluorocycloalkanes such as perfluorocyclobutane, 20 perfluoromethylcyclobutane, perfluorodimethylcyclobutanes, perfluorotrimethylcyclobutanes, perfluorocyclopentane, perfluoromethylcyclopentane, perfluorodimethylcyclopentanes, perfluorocyclohexane, perfluoromethylcyclohexane or 25 perfluorocycloheptane. Other halogenated gases include methyl chloride, fluorinated (e.g. perfluorinated) ketones such as perfluoroacetone and fluorinated (e.g. perfluorinated) ethers such as perfluorodiethyl ether. The use of perfluorinated gases, for example sulphur 30 hexafluoride and perfluorocarbons such as perfluoropropane, perfluorobutanes, perfluoropentanes and perfluorohexanes, may be particularly advantageous in view of the recognised high stability in the blood stream of microbubbles containing such gases. Other 35 gases with physicochemical characteristics which cause them to form highly stable microbubbles in the blood stream may likewise be useful.

Representative examples of contrast agent formulations include microbubbles of gas stabilised (e.g. at least partially encapsulated) by a coalescence-resistant surface membrane (for example gelatin, e.g. as described in WO-A-8002365), a filmogenic protein (for example an albumin such as human serum albumin, e.g. as described in US-A-4718433, US-A-4774958, US-A-4844882, EP-A-0359246, WO-A-9112823, WO-A-9205806, WO-A-9217213, WO-A-9406477, WO-A-9501187 or WO-A-9638180), a polymer material (for example a synthetic biodegradable polymer as described in EP-A-0398935, an elastic interfacial synthetic polymer membrane as described in EP-A-0458745, a microparticulate biodegradable polyaldehyde as described in EP-A-0441468, a microparticulate N-dicarboxylic acid derivative of a polyamino acid - polycyclic imide as described in EP-A-0458079, or a biodegradable polymer as described in WO-A-9317718 or WO-A-9607434), a non-polymeric and non-polymerisable wall-forming material (for example as described in WO-A-9521631), or a surfactant (for example a polyoxyethylene-polyoxypropylene block copolymer surfactant such as a Pluronic, a polymer surfactant as described in WO-A-9506518, or a film-forming surfactant such as a phospholipid, e.g. as described in WO-A-9211873, WO-A-9217212, WO-A-9222247, WO-A-9409829, WO-A-9428780, WO-A-9503835 or WO-A-9729783). Contrast agent formulations comprising free microbubbles of selected gases, e.g. as described in WO-A-9305819, or comprising a liquid-in-liquid emulsion in which the boiling point of the dispersed phase is below the body temperature of the subject to be imaged, e.g. as described in WO-A-9416739, may also be used.

Other useful gas-containing contrast agent formulations include gas-containing solid systems, for example microparticles (especially aggregates of microparticles) having gas contained therewithin or otherwise associated therewith (for example being

adsorbed on the surface thereof and/or contained within voids, cavities or pores therein, e.g. as described in EP-A-0122624, EP-A-0123235, EP-A-0365467, WO-A-9221382, WO-A-9300930, WO-A-9313802, WO-A-9313808 or WO-A-9313809). It will be appreciated that the echogenicity of such microparticulate contrast agents may derive directly from the contained/associated gas and/or from gas (e.g. microbubbles) liberated from the solid material (e.g. upon dissolution of the microparticulate structure). The invention may also be useful in conjunction with contrast agent systems based on microspheres comprising a therapeutic compound as described in e.g. WO98/51284 and WO99/27981.

The disclosures of all of the above-described documents relating to gas-containing contrast agent formulations are incorporated herein by reference.

Gas microbubbles and other gas-containing materials such as microparticles preferably have an initial average size not exceeding 10 μm (e.g. of 7 μm or less) in order to permit their free passage through the pulmonary system following administration, e.g. by intravenous injection. However, larger microbubbles may be employed where, for example, these contain a mixture of one or more relatively blood-soluble or otherwise diffusible gases such as air, oxygen, nitrogen or carbon dioxide with one or more substantially insoluble and non-diffusible gases such as perfluorocarbons. Outward diffusion of the soluble/diffusible gas content following administration will cause such microbubbles rapidly to shrink to a size which will be determined by the amount of insoluble/non-diffusible gas present and which may be selected to permit passage of the resulting microbubbles through the lung capillaries of the pulmonary system.

Where phospholipid-containing contrast agent formulations are employed in accordance with the invention, e.g. in the form of phospholipid-stabilised

gas microbubbles, representative examples of useful phospholipids include lecithins (i.e. phosphatidylcholines), for example natural lecithins such as egg yolk lecithin or soya bean lecithin, 5 semisynthetic (e.g. partially or fully hydrogenated) lecithins and synthetic lecithins such as dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine or distearoylphosphatidylcholine; phosphatidic acids; 10 phosphatidylethanolamines; phosphatidylserines; phosphatidylglycerols; phosphatidylinositols; cardiolipins; sphingomyelins; fluorinated analogues of any of the foregoing; mixtures of any of the foregoing and mixtures with other lipids such as cholesterol. The 15 use of phospholipids predominantly (e.g. at least 75%) comprising molecules individually bearing net overall charge, e.g. negative charge, for example as in naturally occurring (e.g. soya bean or egg yolk derived), semisynthetic (e.g. partially or fully 20 hydrogenated) and synthetic phosphatidylserines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids and/or cardiolipins, for example as described in WO-A-9729783, may be particularly advantageous.

25 Representative examples of materials useful in gas-containing contrast agent microparticles include carbohydrates (for example hexoses such as glucose, fructose or galactose; disaccharides such as sucrose, lactose or maltose; pentoses such as arabinose, xylose 30 or ribose; α -, β - and γ -cyclodextrins; polysaccharides such as starch, hydroxyethyl starch, amylose, amylopectin, glycogen, inulin, pulullan, dextran, carboxymethyl dextran, dextran phosphate, ketodextran, aminoethyldextran, alginates, chitin, chitosan, 35 hyaluronic acid or heparin; and sugar alcohols, including alditols such as mannitol or sorbitol), inorganic salts (e.g. sodium chloride), organic salts

(e.g. sodium citrate, sodium acetate or sodium tartrate), X-ray contrast agents (e.g. any of the commercially available carboxylic acid and non-ionic amide contrast agents typically containing at least one
5 2,4,6-triiodophenyl group having substituents such as carboxyl, carbamoyl, N-alkylcarbamoyl, N-hydroxyalkylcarbamoyl, acylamino, N-alkylacylamino or acylaminomethyl at the 3- and/or 5-positions, as in
10 metrizoic acid, diatrizoic acid, iothalamic acid, ioxaglic acid, iohexol, iopentol, iopamidol, iodixanol, iopromide, metrizamide, iodipamide, meglumine iodipamide, meglumine acetrizoate and meglumine diatrizoate), polypeptides and proteins (e.g. gelatin or albumin such as human serum albumin), and mixtures of
15 any of the foregoing.

The technique described herein would be suitable for use in the infusion of ultrasound products sold under the Trade Names Levovist, Albunex, Optison, Definity, Imagent, Sonovue, Echogen, Sonogen and
20 Sonazoid.

The principles as described herein can obviously be applied to any disperse system where a difference in density leads to segregation (i.e. flotation or sedimentation) over time, such as emulsions, solid
25 particle dispersions and other disperse systems. Preferably, with floating particles the outlet should be near the highest point of the reservoir in order to maintain homogeneity inside the reservoir. Similarly, with sedimenting particles the outlet should preferably
30 be near the low point of the reservoir.

The following non-limitative examples serve to illustrate the invention.

35 Preparation 1 - Hydrogenated phosphatidylserine-encapsulated perfluorobutane microbubbles

Hydrogenated phosphatidylserine (5 mg/ml in a 1% w/w

solution of propylene glycol in purified water) and perfluorobutane gas were homogenised in-line at 7800 rpm and ca. 40°C to yield a creamy-white microbubble dispersion. The dispersion was fractionated to substantially remove undersized microbubbles ($<2\ \mu\text{m}$) and the volume of the dispersion was adjusted to the desired microbubble concentration. Sucrose was then added to a concentration of 92 mg/ml. 2 ml portions of the resulting dispersion were filled into 10 ml flat-bottomed vials specially designed for lyophilisation, and the contents were lyophilised to give a white porous cake. The lyophilisation chamber was then filled with perfluorobutane and the vials were sealed. Prior to use, normally 2 ml water was added to a vial with lyophilised product and the contents were hand-shaken for several seconds to give a perfluorobutane microbubble dispersion with a concentration range of 500-2000 mill. microbubbles/ml ($7\text{-}13\ \mu\text{l/ml}$).

Examples 1-6 - In vitro studies

The contents of a vial prepared as in Preparation 1 were mixed with water (5 ml) from a syringe and gently hand shaken to give a perfluorobutane microbubble dispersion with a microbubble concentration of about $3\ \mu\text{l/ml}$ (Examples 1 to 5). Example 6 describes a procedure in which the contents of a vial prepared as in Preparation 1 were mixed with 2 ml of water. The microbubble dispersion was drawn into a syringe, which was vertically positioned in a module DPC syringe pump and connected to a low volume extension tube equipped with a 3 way stopcock and an administration set for delivery of normal saline from an infusion minibag. The syringe pump rate and the saline rate were varied as shown in Table 1.

Table 1

Example No.	Syringe pump rate (ml/min)	Saline Flush rate (ml/min)	Calculated microbubble concentration (μl/ml)	Observed microbubble concentration (μl/ml)	Infusion steady-state interval (from start of infusion) (min)
5					
1	0.017	1	0.05	0.11±0.04	10-60
2	0.1	1	0.3	0.29±0.05	5-30
3	0.2	1	0.6	0.57±0.06	5-16
10					
4	0.017	2	0.025	0.08±0.01	10-60
5	0.1	2	0.15	0.17±0.03	5-30
6	0.1	2	0.35	0.44±0.04	5-35

Fig. 1 of the accompanying drawings shows a plot of microbubble concentration, expressed as percentage of initial concentration, with time as determined for Example 6. For comparison, the development, with time, of microbubble concentration determined when positioning a syringe horizontally is presented.

It will be appreciated that the length of the administration window will be shortened at higher syringe pump rates given the fixed volume of contrast agent present in a syringe.

Examples 7-14 - In vitro studies

In order to demonstrate the possibility of changing microbubble dose during infusion, a study was conducted using a saline infusion rate of 2 ml/minute, while varying the syringe pump rate as shown in Table 2.

Table 2

	Example No.	Infusion time (minutes)	Syringe pump rate (ml/min)	Calculated microbubble concentration (μl/ml)	Observed microbubble concentration (μl/ml)
5					
	7	5	0.1	0.15	0.20
	8	10	0.05	0.075	0.10
10	9	15	0.05	0.075	0.12
	10	20	0.12	0.18	0.20
	11	25	0.12	0.18	0.19
	12	30	0.05	0.075	0.08
	13	35	0.05	0.075	0.09
15	14	40	0.05	0.075	0.07

Example 15 - In vivo study

Second harmonic imaging of the anterior myocardium was performed on an open chest model in 6 dogs (15-25kg, both sexes) using an ATL HDI 3000 scanner with a mechanical index of 0.6 and 1:4 triggering. Images were recorded following injection of a bolus of contrast agent prepared as in Preparation 1, at a concentration of 30 nl perfluorobutane/kg and during infusion of contrast agent in accordance with the method of the invention, at rates corresponding to 5, 15, 45 and 135 nl perfluorobutane/kg/min. The contrast effects in the region of interest are shown in Fig. 2.

Example 16

A microbubble suspension is prepared as in Example 1 of WO97/48337 and administered according to the method of the invention.

Example 17

5 The commercially available ultrasound product sold under
the Trade name Optison is administered according to the
method of the invention.

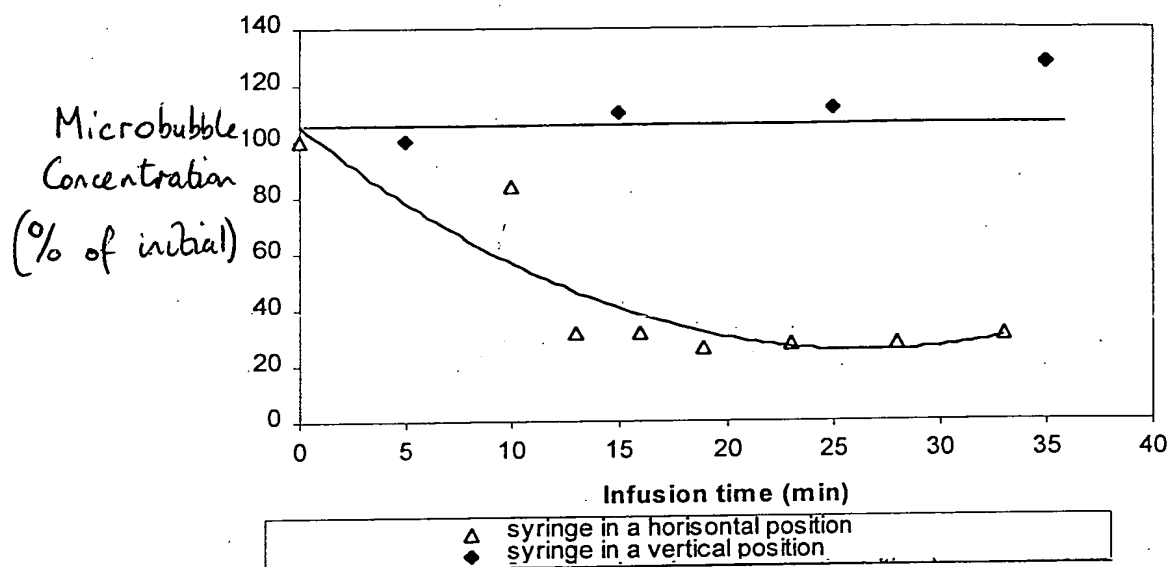
Example 18

10 Contrast agents are prepared as in Example 2 of
WO99/27981 and administered according to the method of
the invention.

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Fig. 1

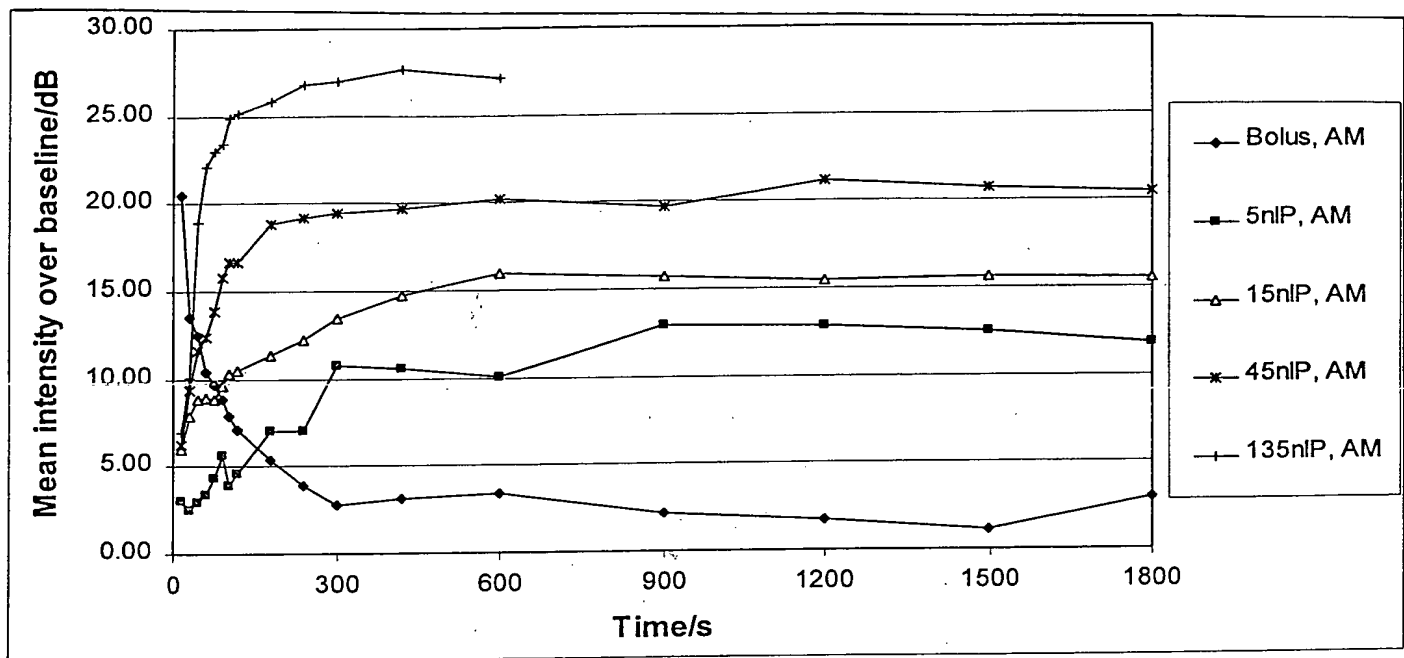


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Fig 2



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